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Production of a soy pastes with "cheese-like" flavor from soy protein and high oleic vegetable oils

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ABSTRACT

We have developed two methods of making cheese-flavored products from soy protein. In the dough method, soy protein (white flakes or soy protein isolate), oil, cheese culture (*Streptococcus salivarius* spp. *thermophilus* and *Lactobacillus delbrueckii* spp. *Bulgaricus*), cheese whey, protease,water and sodium nitrite are mixed and fermented. After the minimal pH is reached, the pH is adjusted to 5.2 with sodium carbonate solution and the cheese is stored anaerobically at 4°C. In the wet method, soy milk (9% solids) is prepared from white flakes, pasteurized, mixed with fat and homogenized. Next, whey, culture and sodium nitrite are added and the mixture is fermented at 40°C. to pH 4.6. The curd is cut and cooked to 50°C, whey is removed and the curd is pressed, adjusted to pH 5.2 with sodium carbonate, protease is added and the product is stored anaerobically at 4°C. The wet method product is a smooth paste. The dough method gave a dryer paste, but it has a much more granular texture. Products made with high-oleic vegetable oils had a bland flavor with little typical soy protein flavor. Products made with milk fat had a pleasant cream cheese-like flavor. The flavor of product made with milk fat could be reproduced in product made with vegetable oils by the addition of a mixture of lactones, ketones and short-chain fatty acids typical of milk fat. The flavor was well developed after one month at 4°C and did not change noticeably with additional storage. The addition of amino acids derived from casein to the products a few days before consumption only marginally improved their cheese-like flavors.

INTRODUCTION

Soy products traditionally were used in many oriental countries because of their nutritional value and low cost. Many clinical studies have shown that consumption of soy products can decrease incidents pf heart diseases, obesity, high blood cholesterol, cancer, diabetes, kidney disease and osteoporosis (Garcia et al., 1997). These health benefits have made soy products more attractive to western consumers. In addition, NASA's interest in using soy products as a major constituent of astronauts' diet on long missions has stimulated research in this area.

The substitution of soy protein in and for dairy products has been explored frequently, including a number of attempts to include soy protein in cheese-like foods. Soy cheese products are present in the market, but the consumption is limited because of poor flavor and texture. Lee (2001) proposed methods for production of spreads with cheese-like flavor from soy protein and milk fat (MF). Earlier, Yu and Hammond (2000) successfully incorporated short-chain fatty acids in high oleic sunflower oil (HOSO) and used the modified oil as a MF substitute for production a Swiss cheese with excellent flavor from skim milk.

We hypothesized that soy spreads with cheese-like flavor could be made by replacing MF with a suitable modification of the vegetable oil. So the objective of this research was production of soy spreads or pastes with cheese-like flavor from soy protein and vegetable oils.

We found that cheese-like flavors in soy pastes made with vegetable oils could be achieved by the use of the free short-chain fatty acids, lactones and ketones that are

characteristics of MF. The interesterificaion of short-chain fatty acids in the vegetable oil also is effective, but since lactones and ketones are necessary for good flavor there is little incentive for this reaction.

LITERATURE REVIEW

Hang and Jackson (1967) were pioneers in the development of methods for soy cheese production. They coagulated soymilk by three methods: 1) addition of calcium sulfate, 2) addition of acetic acid and 3) lactic acid fermentation. The major differences in the cheeses prepared by these three methods were in the yield of precipitated protein, moisture content and hardness. Their results showed that a satisfactory cheese could be prepared by using a lactic acid fermentation of soybean milk.

Tsumura et al. (1976) patented a process for making a cheese-like food from soymilk and a synthetic fat with physical and chemical properties like MF; D-glucono-δ-lactone was used as a soymilk coagulant. They reported that a soy cheese having a flavor profile more closely resembling natural cheese could be obtained by using synthetic fat that contained a group of C4-C10 short-chain fatty acids (SCFA) unique to MF. Later, Tsumura and Hashimoto (1978) patented a method for preparing a firm, sliceable, processed cheese-like product containing no more than 60% (w/w) moisture from soy cheese and a casein-containing material. They suggested the amount of casein-containing material, such as sodium caseinate, calcium caseinate, dairy cheese, dairy cheese curd, whole milk solids, skim milk solids and their mixtures, should be within a range of 1 to 50 parts of casein per 100 parts of soy cheese.

A study on rennet-coagulated curds using 80% (w/w) raw milk and 20% (w/w) soy protein solutions made from defatted soybean meal was investigated by Lee and Marshall (1979). They reported that "the casein curd microstructures were loosened by soy protein, and this caused excessive losses of MF. Soy protein also increased the water holding

capacity of rennet curd". Later, Lee and Marshall (1981) studied the microstructure and texture of process cheese, milk curds and caseinate curds containing native or boiled soy proteins. They reported that soy proteins added to processed cheese, milk curds or caseinate curds were destructive to their microstructure and texture and that soy protein decrease hardness and cohesiveness of the mixtures. They also found that heat denaturated soy protein had a greater destructive effect than native soy protein.

Results similar to Lee and Marshall's were obtained by Abou El-Ella (1980) who worked on a cheese substitute for Egyptian ras cheese using mixtures of soy and cow milk in ratios of 1:3, 1:2, 1:1; *Streptococcus lactis*, 30% (w/w) good quality ripened "ras" cheese solids and calcium lactate. With an increase in soymilk content, the moisture content increased and total nitrogen and fat content were inversely related to soymilk content. He concluded that soymilk could be used as a milk substitute for hard cheese production; however, flavor, body, color and texture improved with the proportion of cow's milk used.

Singh and Mittal (1984) developed a soy cheese spread by blending dehulled and preboiled soybeans and milk solids. They suggested six recipes for a soy cheese spread using rennet and acid fermentation with *S. lactis*. The spreads contained 35% (w/w) solids, 18% (w/w) fat, 11% (w/w) protein and 2% (w/w) sodium chloride. They found that eliminating or limiting soy protein content reduced bitter flavor and the inclusion of skim milk powder improved the flavor of the spreads. Later, Santosh and Singh (1985) studied the effect of processing conditions, starter culture and rennet on flavor development in soy cheese spreads. They found that daily agitation, lactic cultures, rennet and pH 5.3 were essential for good flavor development. A pH of 5.3 prevented sour flavor and improved other

characteristics of the product. Soy slurries with mixed cultures often had off-flavors, but soy slurries with a single culture gave a soy cheese spread with a fresh flavor and clean taste.

Brander et al. (1985) developed a method for making a cheese analog containing soymilk. It was made with 19% (w/w) soymilk, 14% (w/w) to 22% (w/w) vegetable oil, 7% (w/w) to 13% (w/w) dairy whey, 2% (w/w) to 10 % (w/w) caseinate and 38% (w/w) to 58% (w/w) water, plus flavors and colorants. The authors reported good melting and mouthfeel and no off-flavor characteristics in the created product, but cheese-like flavor was not reported.

Rani and Verma (1994, 1995) studied effect of soymilk supplementation on the coagulation time of cheese milk and losses of milk components in whey. Their report agreed with previous studies: cow's milk had a greater content of solids, fat, acidity and ash that soymilk. In blends, increasing soymilk percentage caused coagulation time and yield to increase. The moisture, titratable acidity, soluble protein and free fatty acids of cheese made from cow-soy blends increased with the proportion of the soy solids. Later Singh and Verma (1996) studied cheddar cheese made from cow's milk and from blends with soymilk coagulated by calf rennet and ripened for 240 days. Moisture content decreased with ripening time. The protein, fat, and salt content increased with soy content and were constant on a dry basis with increasing time. During ripening, titratable acidity varied and soluble proteins and fat contents changed slightly.

Chumchuere et al. (2000) developed a method for production of a semi-hard soy cheese. They reported that soy cheese with a moisture content of 61.5% (w/w), a protein content of 21.8% (w/w) and fat content of 2.6% (w/w) did not have an acceptable flavor for

oriental panelists, but when cubes of the soy cheese were deep-fried in corn oil, the hedonic scale improved significantly.

Gottemoller et al. (2002) patented a method for the production by extrusion of fresh soy cheese containing whole milk and dry soy protein isolate. They recommended from 0.92% (w/w) to 2.69% (w/w) of soy isolate addition. Their product had a good texture and milky flavor, but the part of soy protein in that product was low compared to whole milk part.

Han et al. (2002) also attempted to incorporate soy proteins in cheese. Their process used proteinases to treat soy ingredients such as defatted soy flakes, soy protein concentrates, soy protein isolates or soymilk concentrates to form soy protein hydrolyzates. Without the initial enzyme treatment, the intact soy ingredients would interfere with milk clotting, thereby preventing the formation of a normal cheese curd. Cheeses containing up to about 30% (w/w) of partially hydrolyzed soy protein could be made by this method.

Lee (2001) created two methods for cheese-like product production from soy protein and MF. She used fat free soy flakes, MF, 1-2% (w/w) Swiss cheese whey, *Streptococcus thermophilus AC2* and *Lactobacillus delbrueckii var. bulgaricus AR2* as a cheese culture to develop a product with a cheese-spread texture and a pleasant, cheese-like flavor that was free of the beany flavors associated with soy products. This product provided a new option for increasing soy protein consumption and a healthier diet. However it contained 21% (w/w) of MF, which has been identified as a hypercholesterolemic fat because it contains cholesterol and a high percentage of saturated acyl groups (Ney, 1991).

MF has a unique group of C4-C10 SCFA those were reported to have no effect on plasma cholesterol (Jensen et al., 1991). The use of vegetable oils such as high-oleic soybean

(HOSOY) or sunflower oil (HOSO) that are cholesterol-free contain 90% (w/w) of unsaturated acyl groups and are cheaper than MF is an intriguing possibility as a MF substitute. High oleic oils are desirable because the high percentage of polyunsaturates in vegetable oils inhibit dairy cultures (Brady et al., 2003).

However, cheeses made with vegetable oils seem to have oily off-flavors, lower sensory scores and soft textures (Mohamed et al., 1982). One of the problems is that vegetable oils do not have the short-chain acyl groups that are unique to MF that several authors have reported to be important for cheese flavor (Day, 1967; Woo et al., 1982).

Johnson (1991) and Whitehouse (1995) successfully incorporated SCFA in HOSO and made Swiss cheese from such modified high-oleic sunflower oil (MHOSO) and skim milk that had a flavor inferior to that made with MF. However, their method of modification is not acceptable as a commercial food process because a potentially carcinogenic benzene azeotrope was used in the esterification, and toluene sulfonic acid used as a catalyst tends to be partially esterified into the oil (Jiang and Hammond, 2002).

Yu and Hammond (2000) made Swiss type cheese from MHOSO and skim milk that was indistinguishable from that made with MF; they used less toxic toluene azeotrope instead of benzene for HOSO modification but still used toluene sulfonic acid as a catalyst.

A number of studies showed that compounds other than SCFA play an important role in cheese flavor development. Day (1967) reported that methyl ketones and lactones are important components in cheese flavor. Heat treatment of MF saturated with water apparently hydrolyzes 3-keto- and 4- and 5- hydroxy-acyl groups found in MF and γ- and δ-lactones and methyl ketones are formed (Day, 1967).

The objective of this research was the production of soy pastes with cheese like flavor from soy protein and vegetable oils by using suitable modification or additives.

METHODS AND MATERIALS

Ingredients and reagents

Two different types of commercial soy protein were used: soy protein isolate (Protein Technologies International, St. Louis, MO) and fat-free white flakes (Cargill Co., Minneapolis, MN). Casein amino acids were from NZ Amine/Quest Int. (Hoffman Estates, IL). Neutrase 0.8L was from Novo Nordisk Biochem, North America, Inc. (Franklin, NC). High-oleic sunflower oil, Trisun RBD 80, was from ACH Food Co. Memphis, TN. High-oleic soybean oil was provided by DuPont Protein Technologies, St Louis, MO. Other food grade ingredients were obtained from Sigma/Aldrich (Milwaukee, WI) or local groceries. Other reagents and solvents were laboratory grade and obtained from Fisher (Pittsburgh, PA). *Streptococcus thermophilus AC2* and *Lactobacillus delbrueckii var. bulgaricus AR2,* used as cheese cultures, were obtained from the culture collection in the Department of Food Science and Human Nutrition at Iowa State University. *S. thermophilus* was inoculated in 9% (w/w) sterile soymilk and made from fat-free white soy flakes, whereas *L. delbrueckii* was transferred to 9% (w/w) sterile soymilk containing 2% (v/v) liquid cottage cheese whey.

Five types of oils were used: 1) Regular sunflower oil (RSO); 2) High-oleic sunflower oil (HOSO); 3) High-oleic soybean oil (HOSOY); 4) Modified high-oleic sunflower oil (MHOSO) and 5) Milk fat (MF).

Preparation of milk fat

Unsalted sweet cream butter (Crystal Farm, MN, USDA grade AA) was melted at 50°C and centrifuged in 250 ml plastic bottles at 612 * g for 15 min. After centrifugation, the clear upper MF phase was separated from the warm aqueous phase.

Modified high-oleic sunflower oil preparation

MHOSO was prepared by transesterification. The brief vacuum treatment of the vegetable oil and sparging the ethyl esters with nitrogen gas were done before reaction. Ethyl butanoate, hexanoate, octanoate and decanoate were interesterified with Trisun 80 RBD (refined, bleached, deodorized) HOSO (Table 1) with 1.5% (w/w) sodium methoxide powder as a catalyst for 10 h with stirring at 60°C (Table 2).

The completeness of transesterification was monitored by quantitative analysis of the ethyl esters in the reaction mixture by gas chromatography (GC). Acetic acid was added to the transesterification mixture in an amount equimolar with the sodium methoxide. The reaction mixture was washed with water several times until the pH was 6 ±0.5 and filtered through sodium sulfate and Whatman filter #42 using an aspirator and a Buchner funnel. Short-chain ethyl esters exchanged with long-chain glycerol esters of HOSO. Three distillations were done with a Molecular Still (POPE Scientific Inc., Saukville, WI) to remove unwanted long-chain ethyl esters and unreacted short-chain ethyl esters. Distillations conditions are shown in Table 3.

The residue of last distillation was the MHOSO. GC analysis of the MHOSO fatty acid composition was done according to Yu and Hammond (2000a). The short-chain fatty acids composition is shown in Table 4.

Analysis of hydroxy acyl groups in milk fat

Sodium butoxide (2% (w/w) solution in 2.22 g of n-butanol) was prepared by reacting metallic sodium with n-butanol. One gram of MF was reacted with the sodium butoxide solution for 50 min at 40°C. Three drops of acetic acid and 1 ml of hexane were added, and the mixture was washed with water several times until the pH was 6. Each time the interface was preserved with upper phase. The reaction was done in duplicate. After washing, the upper phase was evaporated under a steam of nitrogen to 2.25 ml and 0.30 ml of the sample was applied to a thin-layer chromatography (TLC) plate. The TLC plate with sample was developed with a mixture of 20 ml of freshly distilled diethyl ether and 80 ml of hexane. The plate was sprayed with a 0.5%(w/v) solution of 2',7'-dichlorofluorescein and viewed under ultraviolet light. The TLC separation was done in duplicate. Butyl esters with a hydroxy group R_f=0.29 were lower on the plate than the simple butyl esters R_f=0.88 and were scratched from TLC plate and extracted with 4 ml of methanol and 18 ml of distilled diethyl ether several times. The extracts were centrifuged at 784 * g for 2 min and combined in a 50ml boiling flask. A Boilease fragment was added to the boiling flask, and the ether was distilled gently through a 10-cm vigreux column by placing the boiling flask in a beaker of water at 40°C. The water level in the beaker was kept lower than the liquid level in the boiling flask. When the sample size was reduced to 5 ml the sample was diluted with hexane and analyzed by GC. Twelve commercially available lactones (Sigma/Aldrich, Milwaukee,

WI) were converted to butyl esters to use as external standards using 2% (w/w) sodium butoxide solution in butanol. The amounts of the lactones (L) in MF were calculated using the retention times of external standards to identify the L and the proportions of their peak areas. The results are shown in Table 5. A solution 100 times this concentration was prepared in HOSO for addition to the pastes.

Preparation of the 2-ketones mixture

A 2-ketone mixture as reported in MF by Langer and Day (1964) is given in Table 6. A solution 100 fold more concentrated than Table 6 was prepared in HOSO for addition to the pastes.

Preparation of the free fatty acids mixture

A short-chain free fatty acids (FFA) mixture as reported by Vangtal and Hammond (1986) in ripened Swiss cheese in shown in Table 7. A solution 100 fold more concentrated than Table 7 was prepared in HOSO for addition to the pastes.

Processing of soy pastes by the wet method

Soy pastes made by wet method were prepared according Lee (2001). Fat-free soy white flakes and water in the proportion 9 g flake / 91 g of water were stirred for 2 h and then centrifuged for 1164 * g for 20 min. The centrifuged soymilk was filtered through 4 layers of cheesecloth to remove stray particles, and 2% (w/w) liquid cottage cheese whey was added. The soymilk was pasteurized at 95°C for 7 min. When the pasteurized soymilk

cooled to 55°C, it was mixed with 5% (w/w) fat with a hand blender (Sunbeam, Milford, Mr.) and fed into a two-stage homogenizer (Gaulin-16M, Everett, MA) at 210 kg/cm^2 at the first stage and 70 kg/cm^2 at the second stage. Five kinds of soymilks were prepared with MF, RSO, MHOSO, HOSO and HOSOY. After cooling to 40°C, 0.7%(w/w) of *S.Thermophilus* and 0.3% (w/w) of *L.Bulgaricus* cultures were added to the soymilks, and the milks were incubated at 40°C until the pH of the mixture dropped to about 4.6 and the milks coagulated, which required 6 h for the MF and high-oleic oils but 7.5 h for RSO. The coagulum was cut with a spatula, heated to 50°C and held at this temperature for 30 min. Next sterilized water was used to dilute the curd from 9% (w/w) to 7% (w/w), which reduced the temperature to 45°C. The curd and whey were kept at this temperature for 30 min. The diluted curd was drained through cheesecloth and pressed at 2100 Pa for 6 h. After pressing the pH of the soy paste was adjusted to 5.3 with aqueous saturated sodium carbonate (21.5 g of sodium carbonate in 100 g). The soy paste was blended with 1% (w/w) of salt, 1% (w/w) of cheddar cheese, 1ppm of sodium nitrite, 6.6 ml/100 g of cheese of protease (Neutrase) 0.8 l, Novo Nordisk Biochem, North America, Inc. Franklin, NC) using the KitchenAid blender (Hobart, Troy, OH). For some experiments kid goat lipase (Chr. Hansen, Inc., Milwaukee, WI) was added at 3 different levels (0.025, 0.05 and 0.1% (w/w)) to soy pastes made with MHOSO. Lee (2001) in her work suggested paraffin packaging for soy pastes, but we used vacuum packaging for our product. The soy pastes were transferred to plastic containers, vacuum packed in 51 * 51 cm plastic bags (Koch Supplies Inc., Kansas City, MO) and stored at 4°C. All soy pastes were prepared in duplicate on consecutive days. Some soy pastes were treated with 1% (w/w) of casein amino acids one week before testing by the sensory

panels. Concentrated mixtures of K, L and FFA were added 1h before testing by the sensory panels for experiments which will be indicated later.

Processing of soy pastes by the dough method

Lee (2001) developed a dough method for making soy paste. She used fat-free soy white flakes, but our dough-method soy pastes were prepared using a soy protein isolate. The amount of water was increased 1.4 times compared to Lee's method, and lactose was added as a fermentable sugar source. Soy pastes were prepared using five oils: MF, RSO, MHOSO, HOSO and HOSOY. Soy protein isolate, fat, water, cheese whey, salt, cheddar cheese, protease, lactose, sodium nitrite and culture were mixed in the proportions given in Table 8 and incubated at 40°C until the pH of the mixture dropped to about 5.3, which was 6 h for the MF and high-oleic oils but 8 h for RSO.

As with wet method, vacuum packaging was used instead of the paraffin packaging, as suggested by Lee. The soy pastes were transferred to plastic containers, vacuum packed in the 51 * 51 cm plastic bags (Koch Supplies Inc., Kansas City, MO) and stored at 4°C. All soy pastes were prepared in duplicate on consecutive days. Hydrolyzed casein amino acids 3% (w/w) was added to some soy pastes one week before testing by the sensory panels. Concentrated mixtures of K, L and FFA were added 1h before testing by the sensory panels.

Gas chromatographic analysis

An HP 5890 Series II gas chromatograph (Hewlett-Packer Company) with fused-silica capillary column HP-5 (30m * 0.32 mm i.d., 0.25 μm film thickness) (Hewlett-

Packard, Fisher Scientific Company, Pittsburg, PA) was used. The carrier gas (helium) flow rate was 1.9 ml/min, and the split ratio was 4.54.

The program for ethyl esters content in transesterification mixture was: the oven temperature was held at 30°C for 5 min, programmed to 250°C at 8°C /min and held at 250°C for 5 min. The injector temperature was 220°C, and the detector temperature was 250°C.

The program for FFA composition of MHOSO analysis was: The oven temperature was held at 140°C for 4 min, programmed to 300°C at 5°C /min and held at 300°C for 6 min. The injector temperature was 280°C, and the detector temperature was 300°C.

The program for L butyl esters analysis was: The oven temperature was held at 70°C for 4 min, programmed to 230°C at 10°C /min and held at 230°C for 5 min. The injector temperature was 220°C, and the detector temperature was 230°C.

Chemical analysis

The fat, protein, moisture, salt and pH of the soy pastes were determined in duplicate. Moisture was determined according to method #926.08 (AOAC, 1998). The Pennsylvania Test for Fat from Laboratory Manual: Methods of Analysis of Milk and Its Products (Milk Industry Foundation, 1952) was used for fat content. Protein content in product was measured by the micro-Kjeldahl method with a Tecator Kjeltec 1026 (Höganäs, Sweden). The pH was measured by pH meter (Accumet AR15, Fisher Scientific Company, Pittsburg, PA). The pH meter was calibrated with buffers of pH 7.0 and pH 4.0.

Sensory evaluation

The flavor of soy pastes made by the wet and dough method was evaluated by sensory evaluation using a descriptive analysis. Twelve trained panelists agreed that the flavors noted in representative samples could be described by 10 flavor characteristics: soybeany, rancid, lactone, ketone, cheesy, astringency, bitter, sour, salty and other (Figure 1). The characteristic flavors were quantified using 14-centimeter line scales with the end marked "strong flavor" on the right and "no flavor" on the left end. Panelists were trained in three one-hour sessions before starting the sensory evaluation and retrained when there was a six-week gap in the evaluations. Five-gram samples were served in individual plastic caps. At each testing session, the panelist had five or six samples coded with a three-digit number. Evaluation was done under fluorescent light in individual booths. Panelists tested each set of samples twice with one-day difference between sessions.

Statistical analysis

Data was analyzed using a SPSS 11.5 ANOVA program and Duncan's test.

RESULTS AND DISCUSSION

Oil modification

The goal of our project was to make vegetable oil soy pastes that tasted as good as the soy pastes made with MF by Lee (2001). To accomplish this, we modified vegetable oils to include short-chain esters in concentrations similar to that of MF. Vegetable oil and commercially available ethyl butyrate, caproate, caprylate and caprate were transesterified with a sodium methoxide catalyst. Several problems were encountered in the transesterification. The first problem was excessive soap formation when washing the reaction mixture. Instead of two separated oil and aqueous soap phases, only one gel-like phase was observed. This problem was overcome by removing traces of water from the reaction mixture. This included brief vacuum treatment of the vegetable oil and sparging the ethyl esters with nitrogen gas.

The second problem was that the interesterification seemed to discriminate against incorporation of butyrate into the oil. We solved this problem by increasing amount of ethyl butyrate by 10% (w/w) and stoppering the reaction flask to prevent evaporation of volatile compounds such as ethyl butyrate.

Short- and long-chain ethyl esters remaining or formed in the reaction were removed by short path-distillation using a molecular still. The residue was the MHOSO. The third problem was that, although the SCFA composition of the MHOSO was close to MF, the MHOSO had unpleasant flavor due to undistilled ethyl esters. A three-step distillation solved that problem. The first step was a rapid distillation at 150°C followed by a second distillation at moderate speed and 185.5°C. Finally, a low speed distillation at 200°C

removed remaining ethyl esters and free fatty acids from the MHOSO. After the three-step distillation the MHOSO had a pleasant flavor tested by writer and major professor, and the composition shown in Table 4, which was similar to that of MF.

Free fatty acids

It ultimately seemed more effective to add SCFA typical of MF to pastes as a flavor additive rather than incorporate them into a vegetable oil by interesterification. We used the amounts of these free fatty acids reported in Swiss cheese by Vangtal and Hammond (1986). They are shown in Table 7.

Lactones and ketones

Preliminary experiments, to be discussed later, indicated that MHOSO did not give flavors as good as MF. One hypothesis was that the L and K characteristic of MF were missing. Day (1967) reported that methyl K and L are important components in cheese flavor. Heat treatment of MF saturated with water apparently hydrolyzes 3-keto- and 4- and 5- hydroxy-acyl groups found in MF and γ- and δ-lactones and methyl ketones are formed (Day, 1967). Methyl ketones have been identified in a number of cheese varieties. The complete series of odd-numbered normal methyl ketones from C3 to C15 has been observed in cheddar cheese (Langer and Day, 1964).

A mixture of 2-ketones was prepared according to Langler and Day (1964) is shown in Table 6. They also reported that the maximal amount of methyl ketones was formed in

milk at heated to 140°C. Treatment at lower temperature produced smaller amounts of the ketones.

Quantitative data on L in MF was not found in the literature. MF was converted to butyl esters with n-butanol and sodium butoxide. Thin-layer chromatography was used to separate the butyl esters containing a hydroxy group from normal butyl esters. Figure 2 shows a typical gas chromatogram of the butyl hydroxyesters derived from MF. Twelve commercially available L (Table 5) were converted to butyl esters and used standards to identify the peak in Figure 2 and quantify L. The standard with the longest retention time (16.2 min) was butyl δ-dodecaoate. We ignored L longer than d-dodecalactone, because probably they are not important in cheese flavor, and they are not available commercially. The amount of L determined in 1 g of MF reported in Table 5.

Wet method

In general, the methods for making cheese followed the discoveries of Lee (2001) but several modifications were made. The soymilks with cottage cheese whey were pasteurized at 95°C for 7 min because Lee had found this treatment was necessary to avoid growth of a pink surface mold that contaminated the soy flakes. This treatment also provided an 80% reduction in trypsin inhibitior activity (Bai, 1997). Cottage cheese whey was added to the soymilk before pasteurization because Lee reported that *L. delbrueckii* did not grow in soymilk because of a missing growth factor. This factor could not be supplied by yeast extract or lactose, but the addition of 2% (w/w) of cottage cheese whey enabled the *L. delbrueckii* to multiply well in soymilk.

Pasteurized soymilks with fat incorporated by homogenization was inoculated with cheese culture and incubated at 40°C for ~ 6 h until the pH dropped to 4.6 and the milk to coagulated. When the oil was RSO, we found that 7.5 h was required for the pH to drop to 4.6. Probably this was because of the high content of polyunsaturated acyl groups (Table 1) in RSO. Brady et al. (2003) showed that the polyunsaturates of many vegetable oils inhibit dairy cultures. Sodium nitrite was added as a precaution against *Clostridium botulinum* and other *Clostridia*. Cheddar cheese (1% w/w) was added as a source of unknown cheese microorganisms with the hope that these would make the soy paste have a flavor more like ripened cheese. Numerous studies have suggested that the flavor of ripened cheeses depends on the growth of unknown milk microorganisms as well as the starter culture.

Lee (2001) solved the slight grainy texture problem of soy pastes made by the wet method by the addition of Neutrase, a protease. This addition resulted in a very smooth product texture. The wet method product is a paste because soy protein is more hydrophilic than casein, and its moisture cannot be reduced to that typical of hard cheeses by pressing.

The required pH for a safe cheese with good flavor is in the range of 5.2 to 5.4 Santosh and Singh (1985), so sodium carbonate was added to raise the pH to this range. Lee (2001) reported that a significant pH drop to 4.2 during storage at 4°C was a serious problem with wet method soy pastes. She partially solved this problem by diluting the original soymilk with water from 9% (w/w) to 7% (w/w) solids after cooking, which allowed more of fermentable sugars be removed during pressing. We found this problem could be completely solved by vacuum packaging of the product instead of using a paraffin coating as Lee had done. The pH of the vacuum packaged wet method soy pastes was stable during three mo ripening at 4°C. If the vacuum in the soy pastes was lost, their pH dramatically

dropped from 5.3 to 4.2. Mold growth on the soy pastes surface during ripening period also was avoided by vacuum packaging. If vacuum was lost, the surface of the soy pastes always developed mold.

Soy pastes with cheese-like flavors were prepared by the wet method using five oils: MF, RSO, MHOSO, HOSO and HOSOY.

Dough method

Soy pastes made by dough method by Lee (2001) from fat-free soy white flakes, fat, water, cottage cheese whey, salt, cheddar cheese, protease, sodium nitrite and culture had a very grainy texture, and a pink yeast tended to grow on surfaces exposed to oxygen. Since the production of these pastes did not involve pasteurization, it was difficult to avoid this yeast, and typical yeast inhibitors, such as propionates and sorbic acid, had little effect. Lee (unpublished results) found that irradiation with x-rays would inactive the yeast, but this is not always a convenient or acceptable method.

Lee also suggested adding Neutrase, a protease, for texture improvement, but she reported little effect on the dough method product. As with the wet method sodium nitrite was added as a precaution against *Clostridia*. Cheddar cheese (1% w/w) was added as a potential source of unknown cheese microorganisms that might improve the soy paste's flavor.

We modified Lee's method by using soy protein isolate instead of fat-free soy white flakes to try to improve the texture of dough method pastes. The ingredients and their amounts are shown in Table 8. The soy pastes made with soy isolate had a much better texture compared to those made from the white flakes, but still they did not have as

homogenous a texture as pastes made by the wet method. They were grainy with occasional small lumps. The switch to soy isolate solved the pink yeast problem since the soy isolate seems free of that contaminate.

Lee also had trouble with reproducible pH development using her dough method. As discussed under the wet method, she had found that *L. delbrueckiii* grew poorly on soymilk alone. The addition of 2% (w/w) of cottage cheese whey enabled the *L. delbrueckii* to grow in soymilk, but still 15-25 h were required to reach pH 5.2 in dough method pastes. We solved this problem by increasing the water content to 1.4 times the amount that Lee had used. We also added 0.7% (w/w) of lactose to the soy paste because we expected soy isolate to provide less fermentable sugar than white flakes. Soy paste ingredients and cultures were mixed and incubated at 40°C until the pH of the mixture dropped to about 5.3, which was 6 h for pastes made with MF and high-oleic oils but 8 h for pastes made with RSO. As discussed under the wet method, the slow acid development of pastes made with RSO probably reflects RSO's high content of polyunsaturates. The addition of more moisture and lactose made the fermentation more reproducible and facilitated cheese making.

All dough method pastes were vacuum packaged and had great pH stability and no mold growth during cold ripening period.

Chemical analysis

Chemical composition of soy pastes made by wet method with MF, MHOSO, HOSOY, RSO and HOSO are shown in Table 9 and were not significantly different. All soy pastes made by wet method were uniform, their moisture content ranged from 70.47% (w/w) to 71.02% (w/w), their protein content ranged from 9.16% (w/w) to 9.71% (w/w), their fat

content ranged from 18.45 to 18.85 and their pH range from 5.22 to 5.3. These results were similar to those reported by Lee (2001).

The chemical composition of soy pastes made by the dough method with MF, RSO and HOSO as shown in Table 10 were not significantly different. All soy pastes made by dough method were uniform, their moisture content ranged from 64.8 to 64.91% (w/w), their protein content ranged from 15.80% (w/w) to 16.75% (w/w), their fat content ranged from 17.6% (w/w) to 18.00% (w/w) and their pH ranged from 5.23 (w/w) to 5.28 (w/w).

Preliminary trials and flavor results

Soy pastes made with MHOSO by the wet method had a bland flavor, but, in spite of the short-chain fatty acid composition of the MHOSO, informal evaluations by a panel of five persons agreed that it was not as good as MF. This was surprising because Yu and Hammond (2000b) had found that oils similar to MHOSO emulsified into skim milk and made into Swiss cheese gave excellent flavors. We thought that this might be caused by a lack of lipolysis of the SCFA in our pastes, so, to improve the flavor, kid goat lipase was added at 3 different levels (0.025, 0.05 and 0.1% (w/w)) to wet method soy pastes made with MHOSO. The informal panel evaluated the flavor of the soy pastes made when they were 3, 6 and 8 weeks old. Soy pastes made with MHOSO and 0.1% (w/w) kid goat lipase had a better flavor with 0.1% (w/w) lipase at 3 and 6 weeks than that with 0.025% (w/w) and 0.05% (w/w) lipase. At 8 weeks all the pastes with lipases added had an unacceptable rancid flavor. Although the pastes with lipase had a "cheese-like" flavor, it was more reminiscent of Parmesan, while the pastes made with MF had a mild cream cheese-like flavor.

Seemingly, the MF had flavor compounds not generated in soy pastes that were not available from the MHOSO.

Next for flavor improvement, we tried adding L and K mixture at various concentrations to our product. The informal panel reported that L and K gave soy pastes made with MHOSO a flavor more like those made with MF. Soy pastes with a ketone mixture at the concentration of Table 6 had too strong a blue cheese-like flavor, so lower concentration of ketones in the same proportions as in Table 6 were used. Like K and L added to soy pastes at the concentration of Table 5 gave very strong coconut-like flavors. The L and K were tried at various dilutions of the amounts in Tables 5 and 6 while keeping the relative proportions of the various components constant. The informal panel reported that 25% (w/w) of the tabulated mixture of K and 6.25% (w/w) of the tabulated amounts of L gave the best cheese-like flavors.

It occurred to us that if we must add L and K to get a cream cheese-like flavor in vegetable oil pastes, then we might as well add SCFA instead of going to the trouble of esterifying them in HOSO to make MHOSO. FFA were added to 1-month old wet and dough method soy pastes made with HOSO at levels comparable with that found in Swiss cheese by Vangtal and Hammond (1986) (Table 7). The flavor was compared to pastes made with MHOSO. We found that wet method soy paste made with HOSO and FFA tasted as cheese-like as soy paste made with MHOSO. For the dough method the flavor of paste made with HOSO with FFA was preferred over that made with MHOSO, which had a developed a lipolyzed flavor.

Sensory analysis

To verify the results of the informal panel, we used a larger formal panel and a applied a descriptive sensory analysis technique on pastes made with MF, RSO, MHOSO, HOSO and HOSOY.

Trained panelists selected the flavors "soybeany", "rancid", "lactone", "ketone", "cheesy", "astringent", "bitter", "sour", "salty" and "other" to describe the flavor differences they experience in various soy pastes. The most important of these was the "cheesy", characteristic described the cream-cheese flavor of MF pastes. Soybeany was included to describe flavor perceived as arising from the soy protein. "Rancid" described the lipolyzed flavor imparted to too much short-chain FFA. In samples where too much L and K were added and coconut or blue cheese notes were detected, the terms "lactone" and "ketone," respectively were used. The term "other" was used for any flavor that did not fit into one of the other categories. The remaining terms had their usual flavor meanings.

Wet method sensory results

The flavors of soy pastes made by wet method with MF, RSO, MHOSO, HOSO and HOSOY were evaluated at one month of age and are reported in Table 11. The means for cheesy flavor fell in the order $MF^{c}>MHOSO^{bc}>HOSOY^{ab}>HOSO^{a}>RSO^{a}$ where those with similar superscripts are not significantly different at $p\leq 0.05$. Thus, pastes made with MHOSO were statistically significant like MF, pastes made with RSO, HOSOY and HOSO were significantly different and less "cheesy" than soy pastes made with MF. Soy pastes made with RSO and MF were significantly less sour than other HOSO and HOSOY pastes

($p \leq 0.05$). Soy pastes made with MHOSO were significantly less bitter than pastes made with RSO. None of the other flavor characteristics were significantly different at $p \leq 0.05$.

Next mixtures of L, K and FFA mixtures were tested for their ability to make paste made with HOSO taste like MF and verify our preliminary analyses. The level of FFA was fixed at the levels in Table 7, the level of ketone was 25% (w/w) of that in Table 6, and the level of L was varied from 32% (w/w), 18% (w/w), 6% (w/w) and 2 (w/w) of that in Table 5. In addition, to see if the flavor of the pastes changed significantly during aging, the same samples were evaluated at 1-, 2-, and 3-months of age. The results are shown in Tables 12, 13 and 14. At one month of age, the MF paste was not was not significantly different from that of any of the HOSO samples with L+K+FFA mixtures, but the cheesy flavor scores came closer to the MF score as the L level increased. At two months of age the cheesy flavor of MF^{b}=HOSO+K+FFA+L $18\%^{b\geq}$HOSO+FFA^{ab} $\geq$HOSO+FFA+K+L $6\%^{a}$. At two months of age soy pastes made with HOSO with K, 18% L and FFA added were significantly not different from soy pastes made with MF. Soy pastes made with HOSO with K, 6% L and FFA added were significantly less cheesy than that made with MF or with HOSO with K, 18% L and FFA added. Thus, L6% seemed less concentration of L than needed for two month old soy pastes made with HOSO. Soy pastes made with HOSO with FFA added were not significantly different from other soy pastes. At three months of age the cheesy flavor of MF^{a}=HOSO+K+FFA+L $18\%^{a}$ =HOSO+FFA+K+L $32\%^{a}$. Thus, L18% seemed the optimum level of L, because the soy pastes with that concentrations of L added were not significantly different from soy pastes made with MF at 1-, 2-, and 3-months of age, and the FFA+K+L 18% mixture could make HOSO taste as cheesy as the MF paste. This also showed that

addition of FFA to the soy pastes was a way to have FFA flavor characteristic instead of esterification of short-chain fatty acids ethyl esters in the HOSO.

At two months of age the soy paste made with HOSO+FFA+ 6%LK had significantly more "ketone" flavor than the pastes made with MF and had significantly more "lactone" flavor than pastes made with HOSO+FFA. The soy pastes made with HOSO+ FFA+ 18%LK and with HOSO+FFA were significantly less bitter than pastes made with HOSO+FFA+ 6%LK. Pastes made with HOSO+FFA were less sour than pastes made with HOSO+FFA+6%LK. There were no significant differences among other flavor characteristics. Looking at lower levels of probability for "cheesy" MF was significantly more "cheesy" than HOSO+FFA+6%LK ($p \leq 0.106$) which was less cheesey than HOSO+FFA+ 18%LK ($p \leq 0.246$).

A paste made with MF became contaminated with mold at three months. MF values from the second month were used to compare HOSO with the concentration of L and K used at month two and a L mixture twice as concentrated. None of the flavor characteristics were significantly different at $p \leq 0.05$.

Soy pastes made by the wet method with MF at one and two months and HOSO+ + 18%LK + FFA for 1, 2 and 3 mo of age were compared. The results (Table 15) show that at $p \leq 0.05$ the paste made with HOSO+18%LK +FFA at 3 months was significantly less "cheesy" than MF paste at 1 month, significantly more "soybeany" than HOSO+18%LK +FFA at 2 months and significantly more "salty" than HOSO+18%LK +FFA at 1 and 2 months. HOSO+ 18%LK +FFA at two months was significantly less "bitter" than MF pastes and 1 and 2 months.

Although the paste made with MF became contaminated with mold at three mo, it tasted very good. Its flavor was more like Cheddar or Swiss than the cream cheese-like flavor typical of MF pastes. This observation made us suspect that proteolysis played a role in the development of this flavor and that the cultures we were using were not capable of hydrolyzing soy protein well. Griffith and Hammond (1989) presented evidence that most of the cheese flavor compounds that arise from protein results from the reaction of amino acids with glyoxal, methylglyoxal and dihydroxyacetone produced by the *L. delbrueckii*. So we added amino acids produced by the hydrolysis of casein (CAA) to our MF, HOSO and MHOSO pastes a week before they were tasted. The results were compared with MF, HOSO and MHOSO pastes without CAA at 1 mo of age, and the results are shown in Table 16. MF pastes without CAA and MHOSO pastes with and without CAA were significantly "cheesier" than HOSO pastes with and without CAA and MF with CAA. The HOSO pastes with and without CAA were more "sour" than the MHOSO pastes with and without CAA and MF pastes without CAA. Other flavor characteristics showed no significant differences.

Pastes made with MHOSO, MHOSO+CAA, MHOSO+18%LK, MHOSO+18%LK +CAA and MF were tested at one month of age. The CAA was again added one week before tasting. We found no significant difference in any of the flavor characteristics ($p \leq 0.05$; Table 18).

At $p \leq 0.1$, the soy pastes made with MHOSO with and without CAA were significantly different in "cheesy" flavor from paste made with MF, but the HOSO+18%LK with and without CAA were not significantly different from MF pastes

Results from sensory evaluation analysis of soy pastes made by wet method showed that:

- L+K+FFA mixtures significantly increase "cheesiness" of soy pastes made with HOSO, and 18%LK is close to the optimal concentration of for wet method soy pastes.
- Soy pastes made with MHOSO with 18%LK are not statistically different in flavor to soy pastes made with MF.
- There are no significant difference in flavor found between soy pastes made with HOSO+FFA+18%LK and pastes made with MF.
- CAA significantly decreases cheesiness of soy pastes made with MF and had no significant effect on the cheesiness of HOSO, MHOSO and MHOSO+K+18%LK pastes.
- Aging soy pastes for up to 3 month has little effect on their flavor, possibly because of limited proteolysis.

Dough method sensory results

The flavor evaluation of soy pastes made by dough method was done by the same method, panelists and score sheet as used for the wet method.

Although HOSO pastes had proven to be superior to RSO pastes in the wet method, we tested them again in the dough method at one month with and without added CAA. The results are shown in Table 18. The unexpected result for cheesiness were that the $RSO+CAA^{b} \geq RSO^{ab} \geq HOSO+CAA^{a}=HOSO^{a}$. Thus, RSO was not significant cheesier than HOSO and CAA made RSO significantly cheesier than HOSO and HOSO+CAA. This result was surprising because earlier work suggested high-oleic oils gave cheesier flavor than

vegetable oils rich in linoleate (Johnson, 1991, Whitehouse, 1995, Yu, 2000). Pastes made with RSO+CAA were significantly more "salty" than pastes made with HOSO. Soy pastes with CAA were scored significantly higher in "other" flavor, probably because panelists noticed the CAA flavor but did not identify it as "cheesy," and this was one of the panel's first experience in scoring samples with CAA.

Next one month-old pastes made with RSO+K+FFA and with L at two percentages was compared with MF (Table 19). At the 6% (w/w) and 18% (w/w) levels of L, the RSO+K+FFA+L scored cheesier than the MF paste but the difference was significant only for the 18% (w/w) level. The cheesiness of the MF sample in this comparison scored unusually low. At 2 mo, cheesy flavor of pastes made with RSO+K+FFA and with 18% (w/w) and 32% (w/w) levels of L was scored higher (Table 20) but not significantly different from the RSO+K+FFA+18%L paste. CAA did not improve the cheesiness of the RSO+K+FFA+18%L paste.

Data from samples common to Table 19 and 20 were combined to look for differences caused by samples age (Table 21).

At $p \leq 0.05$, the flavor of pastes made with MF and RSO+18%L+K+FFA+CAA were not significantly different in any flavor attribute at 1 and 2 mo age. Pastes made with MF at one month were significantly less cheesy at $p \leq 0.05$ from ROS+18%L +FFA+CAA at one month.

Results from the sensory evaluation of soy pastes made by dough method showed that:

- Soy pastes made with RSO are significantly cheesier than those made with HOSO.

- It is possible that CAA added to soy pastes increase cheesiness, but in our case it was not a significant effect.
- L+K+FAA mixtures significantly increase cheesiness of soy pastes and 18% (w/w) L is approximately the optimum concentration.
- The flavor of soy paste made with RSO+K+18%L +FFA+CAA was not significantly different from that made with MF.
- The flavor of soy paste made with MF and RSO+18%L +FFA+CAA does not change significantly between one and two months age.

CONCLUSIONS

We have been able to achieve cheese-like flavors in soy pastes made with vegetable oils comparable to those produced by using MF by the use of FFA, L and K additives. This is more feasible in space then the complex interesterification of FFA into vegetable oils followed by molecular distillation. But, the addition of flavor additives about 30 min before consumption is necessary. If the ingredients are put in too soon, the cultures will probably consume them (Yu and Hammond, 2000b). If one does not wait for 30 min after thorough mixing, the flavor will not be properly distributed.

The flavors obtained are reminiscent of dairy products but are more like cream cheese than a typical ripened cheese. It appears that the lipolized flavors typical of parmesan could be produced by the inclusion of greater concentrations of FFA. The attainment of a more robust flavor like that of Cheddar will depend on achieving a greater degree of proteolysis by selection of an organism or enzyme. In the meantime, it is doubtful if the addition of Cheddar cheese to the pastes as a source of flavor-generating cultures makes any difference. The use of whey to stimulate the growth of *L. delbreuckii* could be eliminated by the identification of the necessary growth factor. The pure factor would probably require much less weight, which is an important consideration in space.

The texture of pastes made by the dough method might be improved by a machine that would homogenize such thick pastes. The achievement of a more cheese-like texture by reducing the water content seems to be impossible because soy protein is more hydrophilic than is casein. But a sliceable version of our pastes could undoubtedly be produced by the introduction of a suitable gelling agent.

TABLES AND FIGURES

Table 1. Fatty Acid Composition and Peroxide Value of Regular Sunflower Oil (RSO), High Oleic Sunflower Oil (HOSO) and High Oleic Soybean Oil (HOSOY).

	C16:0 % (w/w)	C18:0 % (w/w)	C18:1 % (w/w)	C18:2 % (w/w)	C18:3 % (w/w)	Peroxide Value
HOSO	5.8	5.2	82.4	6.7	0.5	0.8
HOSOY	5.6	2.5	86.8	3.9	2.5	0.84
RSO	6.1	5.3	20.5	66.5	1	0.93

Table 2. Amounts of Short-Chain Esters and HOSO Used for Transesterification.

	Ethyl butyrate % (w/w)	Ethyl Caproate % (w/w)	Ethyl Caprylate % (w/w)	Ethyl Capriate % (w/w)	HOSO % (w/w)
Reaction Mixture	4.25	3.28	1.60	3.35	87.50

Table 3. Distillations Conditions Used to Remove Ethyl Esters from MHOSO and MHOSOY

	Temperature, °C	Speed, drops/sec	Pressure, torr
1st distillation	150	5	0.08
2nd distillation	185	2	0.06
3rd distillation	200	1	0.02-0.04

Table 4. The Short-Chain Fatty Acid Composition of MHOSO and the Amount Found in a Typical Milk Fat.

	C4:0 % (w/w)	C6:0 % (w/w)	C8:0 % (w/w)	C10:0 % (w/w)
MHOSO	2.8	2.5	1.6	2.7
MF	2.6	2.4	1.6	2.8

Table 5. Amounts of Lactones Found in 1 g of Milk Fat.

L	γC_5	γC_6	δC_6	γC_7	γC_8	γC_9	δC_9	γC_{10}	δC_{10}	γC_{11}	δC_{11}	γC_{12}
ppm	2	28	5	9	55	10	15	14	176	19	35	300

Table 6. Composition of the 2-Ketone Mixture Added to Soy Pastes.

K	C_3	C_4	C_5	C_7	C_9	C_{11}	C_{13}
mg / 100 g of paste	1.4	1.3	2.0	3.8	1.8	2.0	4.6

Table 7. Composition of the Free Fatty Acids Mixture Added to Soy Pastes.

FFA	C4:0	C6:0	C8:0	C10:0
mg/100 g of Paste	94	36	11	7

Table 8. Amount of Ingredients Used in the Dough Method for Soy Pastes.

Ingredient	**Amount, % (w/w)**
Soy protein isolate	26
Fat	16
Water	52
Cottage cheese whey	2
Salt	1
Protease (Neutrase 0.8 L, Novo Nordisk Biochem, North America, Inc. Franklin, NC)	*
Lactose	0.7
Cheddar cheese	1
Sodium nitrite	**
Streptococcus thermophilus AC2 culture	0.7
Lactobacillus delbrueckii var. bulgaricus AR2 culture	0.3

* *protease was added as 6.6 μL/100 g of soy paste.*

** *sodium nitrite was added as 1 ppm.*

Table 9. Chemical Composition of Various Soy Pastes Made by the Wet Method.

Fat used	Fat % (w/w)	Protein % (w/w)	Mousture % (w/w)	pH
MF	18.70a	9.25a	70.47a	5.30a
RSO	18.50a	9.16a	70.68a	5.32a
HOSO	18.50a	9.38a	70.63a	5.28a
HOSOY	18.85a	9.71a	70.37a	5.22a
MHOSO	18.45a	9.42a	71.02a	5.30a

Means with different letters within a row differ at $p \leq 0.05$.

Table 10. Chemical Composition of Various Soy Pastes Made by Dough Method.

Fat used	Fat % (w/w)	Protein % (w/w)	Moisture % (w/w)	pH
MF	18.00a	16.75a	64.80a	5.23a
RSO	17.60a	15.80a	64.82a	5.28a
HOSO	17.90a	16.30a	64.91a	5.25a

Means with different letters within a row differ at $p \leq 0.05$.

Table 11. Sensory Means* of 1-Month Old Soy Pastes Made by the Wet Method.

Flavor characteristic	MF	RSO	MHOSO	HOSO	HOSOY
Soybeany	0.67a	0.88a	0.90a	1.04a	0.67a
Rancid	0.63a	0.83a	0.66a	0.55a	0.63a
Lactone	1.67a	0.94a	2.16a	1.30a	1.44a
Ketone	2.09a	1.78a	1.95a	1.17a	1.54a
Cheesy	5.91c	3.48a	5.61bc	4.08a	4.51ab
Astringent	1.30a	1.58a	1.12a	1.59a	2.03a
Bitter	1.42ab	2,55b	1.18a	1.96ab	1.83ab
Sour	1.76a	1.70a	2.33ab	3.32b	3.32b
Salty	1.18a	2.35a	1.22a	1.68a	1.64a
Other	0.79a	1.77ab	1.22a	2.48b	1.04a

* *Flavor characteristics were determined 1 h after removal from storage at 4°C by 12 trained panelists using a 14 cm line scales from 0 cm (no flavor) to 14 cm (strong flavor). Panelists tested each set of samples twice with one-day difference between sessions. Means with different letters within a row differ at $p \leq 0.05$.*

Table 12. Sensory Means of 1-month Old Soy Pastes Made by Wet Method.

Flavor characteristic	MF	HOSO 2% (w/w) L,K,FFA	HOSO 6% (w/w) L,K,FFA	HOSO 18% (w/w) L,K,FFA
Soybeany	0.60a	0.77a	0.76a	1.1a
Rancid	0.74a	0.64a	0.79a	0.58a
Lactone	1.75a	1.81a	2.19a	1.83a
Ketone	2.96a	2.08a	2.71a	1.91a
Cheesy	6.07a	5.01a	5.34a	5.53a
Astringent	2.26a	2.66a	2.28a	2.44a
Bitter	2.03a	2.40a	2.36a	2.28a
Sour	3.37a	2.55a	2.93a	3.17a
Salty	1.78a	2.64a	2.67a	1.83a
Other	1.76a	1.42a	1.32a	1.69a

* *Flavor characteristics were determined 1 h after removal from storage at 4°C by 12 trained panelists using a 14 cm line scales from 0 cm (no flavor) to 14 cm (strong flavor). Panelists tested each set of samples twice with one-day difference between sessions. Means with different letters within a row differ at $p \leq 0.05$.*

Table 13. Sensory Means of 2-month Old Soy Pastes Made by Wet Method with HOSO and MF.

Flavor characteristic	MF	HOSO FFA	HOSO 6% (w/w) L,K,FFA	HOSO 18% (w/w) L,K,FFA
Soybeany	0.76a	0.22a	0.37a	0.35a
Rancid	0.69a	0.55a	0.47a	0.48a
Lactone	1.33ab	0.58a	2.31b	1.62ab
Ketone	1.46a	2.94ab	3.46b	2.91ab
Cheesy	5.95b	5.42ab	4.33a	5.94b
Astringent	1.90a	1.07a	1.70a	1.68a
Bitter	1.67ab	0.72a	2.27b	0.65a
Sour	3.57c	1.82a	3.27bc	2.25ab
Salty	1.33a	1.55a	1.71a	1.30a
Other	2.14a	1.63a	2.10a	1.76a

* *Flavor characteristics were determined 1 h after removal from storage at 4°C by 12 trained panelists using a 14 cm line scales from 0 cm (no flavor) to 14 cm (strong flavor). Panelists tested each set of samples twice with one-day difference between sessions. Means with different letters within a row differ at $p \leq 0.05$.*

Table 14. Sensory Means* of 3-month Old Soy Pastes Made by Wet Method with HOSO and MF.

Flavor characteristic	MF2	HOSO 18% (w/w) L,K,FFA	HOSO 32% (w/w) L,K,FFA
Soybeany	0.76a	1.34a	1.33a
Rancid	0.69a	0.95a	1.25a
Lactone	1.33a	2.13a	2.29a
Ketone	1.46a	1.76a	2.24a
Cheesy	5.95a	4.50a	4.66a
Astringent	1.90a	1.44a	1.18a
Bitter	1.67a	1.34a	1.43a
Sour	3.57a	2.86a	3.33a
Salty	1.33a	2.44a	2.94a
Other	2.14a	1.20a	1.44a

* *MF3 was moldy and MF2 was used in the comparisons. Flavor characteristics were determined 1 h after removal from storage at 4°C by 12 trained panelists using a 14 cm line scales from 0 cm (no flavor) to 14 cm (strong flavor). Panelists tested each set of samples twice with one-day difference between sessions. Means with different letters within a row differ at* $p \leq 0.05$.

Table 15. Sensory Means* of 1, 2 and 3-month Old Soy Pastes Made by Wet Method.

Flavor characteristic	MF1	MF2	HOSO1 18% (w/w) L,K,FFA	HOSO2 18% (w/w) L,K,FFA	HOSO3 18% (w/w) L,K,FFA
Soybeany	0.60ab	0.76ab	1.10ab	0.35a	1.41b
Rancid	0.74a	0.69a	0.58a	0.48a	0.95a
Lactone	1.75a	1.33a	1.83a	1.62a	2.13a
Ketone	2.96a	1.46a	1.91a	2.91a	1.76a
Cheesy	6.07b	5.95ab	5.53ab	5.94ab	4.50a
Astringent	2.26a	1.90a	2.44a	1.68a	1.44a
Bitter	2.03b	1.67ab	2.28b	0.65a	1.34ab
Sour	3.37a	3.57a	3.17a	2.25a	2.86a
Salty	1.78ab	1.33a	1.83ab	1.30a	2.44b
Other	1.76a	2.14a	1.69a	1.76a	1.20a

* *The number after oil refers to the age of the sample in months. Flavor characteristics were determined 1 h after removal from storage at 4°C by 12 trained panelists using a 14 cm line scales from 0 cm (no flavor) to 14 cm (strong flavor). Panelists tested each set of samples twice with one-day difference between sessions.Means with different letters within a row differ at $p \leq 0.05$.*

Table 16. Sensory Means of 1-month Old Soy Pastes Made by Wet Method with MF, MHOSO and HOSO with and without CAA.

Flavor characteristic	MF	MF CAA	MHOSO	MHOSO CAA	HOSO	HOSO CAA
Soybeany	0.95a	0.74a	0.94a	0.74a	1.02a	1.08a
Rancid	0.30a	0.75a	0.52a	0.63a	0.64a	0.82a
Lactone	0.83a	0.81a	1.05a	1.25a	1.09a	1.40a
Ketone	1.05a	0.73a	0.88a	2.07a	1.30a	1.25a
Cheesy	6.28b	3.65a	5.84b	5.80b	3.34a	3.43a
Astringent	0.89a	1.20a	0.82a	0.60a	1.25a	1.82a
Bitter	1.36a	1.80a	1.38a	1.31a	1.70a	1.97a
Sour	1.56a	3.00ab	1.90a	2.12a	4.02b	4.19b
Salty	0.79a	1.12a	1.13a	1.95a	1.39a	1.57a
Other	0.75a	2.27a	1.50a	1.69a	2.76a	2.77a

* *Flavor characteristics were determined 1 h after removal from storage at 4°C by 12 trained panelists using a 14 cm line scales from 0 cm (no flavor) to 14 cm (strong flavor). Panelists tested each set of samples twice with one-day difference between sessions. Means with different letters within a row differ at $p \leq 0.05$.*

Table 17. Sensory Means of 1-month Old Soy Pastes Made by Wet Method with MF and MHOSO.

Flavor characteristic	MF1	MHOSO1 18% (w/w) L,K	MHOSO1 18% (w/w) L,K,CAA	MHOSO1 CAA	MHOSO1
Soybeany	0.51a	0.81a	0.85a	0.79a	0.91a
Rancid	0.13a	0.30a	0.51a	0.53a	0.57a
Lactone	1.05a	1.15a	2.14a	1.62a	1.89a
Ketone	1.60a	1.24a	1.25a	0.99a	1.90a
Cheesy	6.29b	5.2ab	4.94ab	4.80a	4.58a
Astringent	0.90a	0.88a	1.11a	1.40a	1.00a
Bitter	0.71a	1.09ab	1.45ab	1.11ab	1.96b
Sour	2.25a	2.68a	2.30a	2.02a	3.27a
Salty	0.91a	0.85a	0.78a	1.18a	0.70a
Other	1.34ab	0.76a	2.30b	1.31ab	1.20ab

* *Flavor characteristics were determined 1 h after removal from storage at 4°C by 12 trained panelists using a 14 cm line scales from 0 cm (no flavor) to 14 cm (strong flavor). Panelists tested each set of samples twice with one-day difference between sessions. Means with different letters within a row differ at $p \leq 0.05$.*

Table 18. Sensory Means of 1-month Old Soy Pastes Made by Dough Method.

Flavor characteristic	HOSO	RSO	HOSO+CAA	RSO+CAA
Soybeany	3.18a	1.79a	2.11a	1.93a
Rancid	0.86a	0.90a	0.44a	0.93a
Lactone	1.11a	0.95a	1.07a	1.14a
Ketone	1.38a	1.63a	1.49a	1.64a
Cheesy	2.00a	3.35ab	2.16a	4.40b
Astringent	1.39a	1.69a	1.73a	2.15a
Bitter	0.70a	1.03a	1.13a	1.45a
Sour	1.60a	2.54a	1.89a	2.40a
Salty	1.65 a	2.22 ab	2.36 ab	3.51 b
Other	1.14 a	1.31 a	4.35 b	3.71 b

Flavor characteristics were determined 1 h after removal from storage at 4°C by 12 trained panelists using a 14 cm line scales from 0 cm (no flavor) to 14 cm (strong flavor). Panelists tested each set of samples twice with one-day difference between sessions. Means with different letters within a row differ at $p \leq 0.05$.

Table 19. Sensory Means of 1-month Old Soy Pastes Made by Dough Method.

Flavor characteristic	MF	RSO 6% (w/w) L,K,FFA,CAA	RSO 6% (w/w) L,K,FFA	RSO 18% (w/w) L,K FFA,CAA
Soybeany	1.07a	1.70a	1.79a	1.56a
Rancid	0.50a	1.14a	0.89a	0.94a
Lactone	0.71a	1.19a	1.38a	2.00a
Ketone	1.31a	1.01a	1.04a	1.39a
Cheesy	2.52a	3.81ab	3.92ab	4.31b
Astringent	1.63a	2.08a	2.04a	2.20a
Bitter	0.67a	1.42a	1.44a	1.20a
Sour	1.48a	2.61ab	2.78b	2.56ab
Salty	1.27a	2.37a	2.83a	2.58a
Other	3.56a	1.77a	2.26a	2.17a

* *Flavor characteristics were determined 1 h after removal from storage at 4°C by 12 trained panelists using a 14 cm line scales from 0 cm (no flavor) to 14 cm (strong flavor) Panelists tested each set of samples twice with one-day difference between sessions. Means with different letters within a row differ at $p \leq 0.05$.*

Table 20. Sensory Mean of 2-month Old Soy Pastes Made by Dough Method with RSO and MF.

Flavor characteristic	MF	RSO 18% (w/w) L,K,FFA,CAA	RSO 18% (w/w) L,K,FFA	RSO 32% (w/w) L,K,FFA,CAA
Soybeany	1.33a	1.28a	2.11a	1.49a
Rancid	0.83a	0.68a	1.15a	0.74a
Lactone	1.05a	1.18a	0.99a	1.61a
Ketone	1.27a	1.38a	1.37a	1.61a
Cheesy	3.96a	3.50a	3.40a	3.06a
Astringent	1.35a	2.10a	1.65a	1.84a
Bitter	0.73a	1.16a	1.00a	1.23a
Sour	2.03a	2.41a	2.39a	1.85a
Salty	2.06a	2.55a	1.23a	2.29a
Other	1.86a	2.91a	2.02a	2.46a

* *Flavor characteristics were determined 1 h after removal from storage at 4°C by 12 trained panelists using a 14 cm line scales from 0 cm (no flavor) to 14 cm (strong flavor). Panelists tested each set of samples twice with one-day difference between sessions. Means with different letters within a row differ at $p \leq 0.05$.*

Table 21. Sensory Means* of 1 and 2-month Old Soy Pastes Made by the Dough Method.

Flavor characteristic	MF1	MF2	RSO1 18% (w/w) L,K,FFA,CAA	RSO2 18% (w/w) L,K,FFA,CAA
Soybeany	1.07a	1.33a	1.56a	1.28a
Rancid	0.51a	0.83a	0.94a	0.68a
Lactone	0.71a	1.05a	2.00a	1.18a
Ketone	1.31a	1.27a	1.40a	1.38a
Cheesy	2.52a	3.96ab	4.31b	3.51ab
Astringent	1.63a	1.35a	2.20a	2.10a
Bitter	0.67a	0.73a	1.20a	1.16a
Sour	1.47a	2.03a	2.56a	2.42a
Salty	1.27a	2.06a	2.58a	2.55a
Other	3.56a	1.86a	2.17a	2.91a

* *The number after oil refers to the age of the sample in months. * Flavor characteristics were determined 1 h after removal from storage at 4°C by 12 trained panelists using a 14 cm line scales from 0 cm (no flavor) to 14 cm (strong flavor). Panelists tested each set of samples twice with one-day difference between sessions. Means with different letters within a row differ at $p \leq 0.05$.*

Figure 1. A sample soy cheese sensory evaluation form.

Soycheese sensory evaluation

Name:_____
Date:_____

You are receiving samples of soy cheese.
Evaluate these samples for flavor. Test each sample.

Soybeany

no flavor ——————————————— strong flavor

Rancid

no flavor ——————————————— strong flavor

Lactone/Coconut

no flavor ——————————————— strong flavor

Ketone/Blue cheese

no flavor ——————————————— strong flavor

Cheesy

no flavor ——————————————— strong flavor

Astringent

no flavor ——————————————— strong flavor

Bitter

no flavor ——————————————— strong flavor

Sour

no flavor ——————————————— strong flavor

Comments:

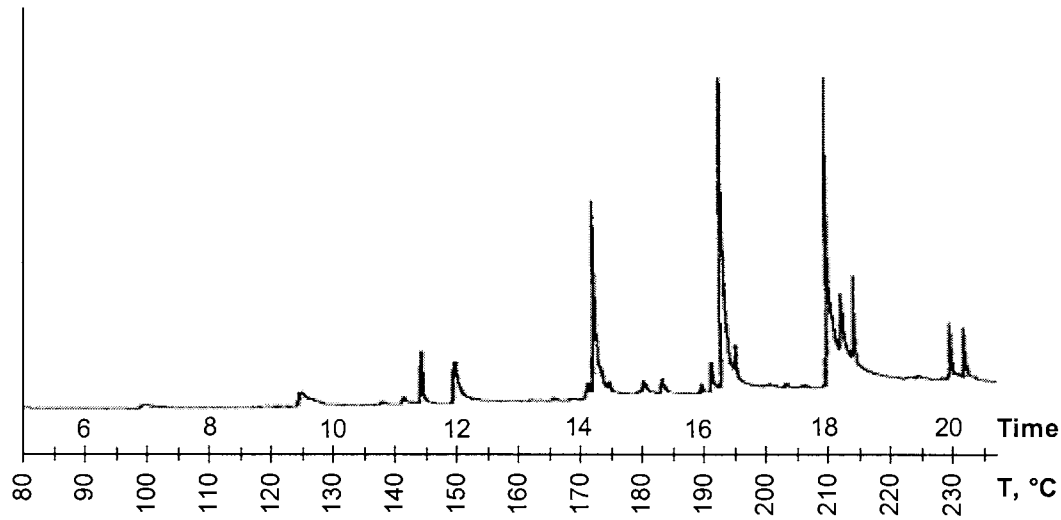

Figure 2. Gas Chromatogram of Butyl Esters of Hydroxy Acyl Compounds from MF

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